Editorial

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Will nano-fibers permit to turn liver cell transplantation into a curative tool against liver failure?

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The article by Navarro-Alvarez and colleagues, entitled "Intramuscular transplantation of engineered hepatic tissue constructs corrects acute and chronic liver failure in mice" [1] and published in the current issue of Journal of Hepatology, offers an interesting approach towards the future of cell transplantation and tissue engineering. These authors investigated whether a non-immnunogenic self-assembling peptide nano-fiber (SAPNF), used in combination with conditioned media (CM) derived from an immortalized human hepatocyte cell line, was capable of providing a three dimensional scaffold that allowed hepatocytes to maintain their function. The experiments were performed in culture and *in vivo* after transplantation of engineered tissue within the skeletal muscle of mice, and the results compared to cells grown in Matrigel, an extracellular matrix mimicking the physiological growth microenvironment of hepatocytes. A first set of experiments demonstrated that cells grown in SAPNF/CM matrix kept a mature hepatocyte phenotype, expressing albumin and cytochrome, and showed signs of de-differentiation such as expression of CK19 and AFP after 21 days in cultures [1]. SAPNF/CM engrafted cells harboured major detoxification properties, and metabolized ammonia, lidocaine, and diazepam more effectively than cells grown in Matrigel. Another set of experiments using *in vivo* models of fulminant and chronic hepatitis clearly established the superiority of SAPNF/CM engrafted hepatocytes in terms of overall survival rate, decrease of ammonia levels and encephalopathy [1]. The original locus (within the skeletal muscle) for cell implantation tested out by the authors allowed a large number of cell transplantations without the risk of portal hypertension, thrombosis and pulmonary embolism associated with intraportal infusion [2]; this definitely further improved the quality of the results. On the whole, this work proposes an elegant and a relatively safe therapeutic procedure that may be easily scaled up to clinic as an alternative to liver replacement strategies. A further advantage of polymeric matrices with regard to cell transplantation is that it provides a reversibility of the surgical act, due to the fact that the transplant can be easily removed.

Until now, orthotopic liver transplantation has remained the ultimate life-saving therapy for numerous end-stage liver diseases such as fulminant or chronic hepatitis and inborn metabolic disorders. Besides the requirement of a surgical team with an expertise for this procedure, the shortage in transplantable material still limits the organ replacement strategy, which only fulfills about 20% of the needs. This has raised an interest in developing alternative approaches to support liver functions, in order to give a chance for the liver to recover or to stretch out the life expectancy of patients waiting for a donor liver. Among these approaches, cell and tissue engineered transplantation have yielded promising results. In recent years, cell transplantation has been a particularly active area with numerous proofs of concept generated in animal models as well as patients with acute or chronic liver failure and metabolic disorders (for review see [3,4]). In the literature, up to 40 patients have been reported to undergo cell transplantation treatment after having been diagnosed with acute liver failure and multiple organ failure – arising from different aetiologies – and exhibited encouraging results with regard to ammonia levels as well as measurable improvements of encephalopathy. Cell transplantation successfully bridged the 2–10 day time lag patients have to wait for liver transplants. Similarly, encouraging results have also been reported for patients with chronic liver failure, with more than 20 patients treated so far. Patients bore decreased ammonia levels and controlled encephalopathy for 6 weeks after a single infusion, and for 6 months after 10 direct intrasplenic transplantations (for review [3]). Hepatocyte transplantation has also demonstrated its efficiency in correcting metabolic disorders, using either allotransplantation [5], or genetically modified autologous hepatocytes [6]. The potential advantages of the cell-transplantation technology include a relatively low cost, a demonstrated feasibility, and the need for a relatively small number of hepatocytes; these cells are isolated from untransplantable human liver segments and are either directly infused in the portal vein or spleen, or cryopreserved until needed. Moreover, alternative approaches in using hepatic progenitors [7], bone marrow stem cells [8], *in vitro* expansion of hepatocytes or immortalized hepatocytes [9] are under investigation. However, the relative capability of these cell types to engraft and repopulate the liver has not yet been assessed. To date, mature hepatocytes appear to be

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more efficient than progenitors or stem cells for liver repopulation [7]. In addition, immortalized hepatocytes display karyotypic instability over time of culture, thus limiting their clinical use [10].

Hepatocyte transplantation is likely to become a valuable clinical tool to improve the treatment of liver failure. Currently, its main limitation as a curative treatment of chronic and metabolic disorders concerns the long term survival of differentiated hepatocytes after cell transplantation, as this is a necessary requirement for the maintenance of specific liver functions. Hepatocytes are attachment-dependent cells and degenerate rapidly without optimal media, extracellular matrix and cell–cell contact. To survive, they need proper microenvironmental structures and signals. Moreover, as pointed out by Navarro-Alvarez and colleagues in this issue of Journal of Hepatology [1], cirrhotic patients harbour altered architectures that are not considered as appropriate for hepatocyte transplantation. To solve these issues, cell transplantation needs to evolve towards transplantable tissue engineering.

Transplantations of engineered hepatic tissue using different polymer structures have been reported before. Kim and colleagues demonstrated *in vitro* the long term survival of hepatocytes in polymer scaffold in 1998 [11], followed in 1999 by Kaufmann et al. reporting cell survival after transplantation of a polymeric structure in rats [12]. In 2006, Yokoyama and colleagues assayed a bFGF-releasing polymer that allowed hepatocyte survival in the subcutaneous cavity for up to 120 days [13]. Recently, Soto-Gutierrez and colleague reported that 60% of mice survived after 30 days following 90% hepatectomy, while 0% survived in the control group (implantation of the 3D matrix without hepatocytes). This establishes the efficiency of this technology in improving mice survival after 90% hepatectomy [14]. The paper by Navarro-Alvarez and colleagues goes further in establishing the *in vitro* and *in vivo* proof of concept, as it is one of the few if not unique works presenting the advantage of tissue engineered replacement strategy in both acute and chronic liver failure [1].

Two other strategies are currently under investigation, either as a support or as a replacement for liver transplantation interventions, namely extracorporeal bioartificial livers and extracorporeal liver assisted devices. Bioartificial liver is an external bioreactor containing living hepatocytes and allowing patient bloodstream to go through for detoxification [15]. Several clinical trials were carried out using such devices, with either hepatocytes from porcine [16], and human [17] origin, or hepatoma-derived cells [17]. These studies showed the safety of the devices on patients with fulminant hepatic failure; they decreased bilirubin and ammonia levels, and to some extent, provided survival benefit. The main problem associated with the clinical use of these bioreactors is the scale-up requirement of a device containing at least 10^{10} live hepatocytes. The extracorporeal liver assist devices are an outcome of the recent evolution of various dialysis systems in use since the early 1970s and are based on the scavenging power of albumin. Albumin dialysis against albumin-containing solution across a highly permeable high-flux membrane gave rise to several devices such as the Molecular Adsorbent Recirculation System (MARS), the Single-Pass Albumin Dialysis (SPDA), and the Fractionated Plasma Separation and Adsorption (FPSA) also called Prometheus. Most clinical studies, performed in acute liver failure or in acute chronic liver failure, demonstrated biochemical improvements in terms of bilirubin, bile acids, creatine, and ammonia levels [18,19]. For patients with an acute chronic liver failure, these improvements were accompanied with a decrease in hepatic encephalopathy grade and, in some cases, a slight survival benefit [19,20]. However, it is still unclear whether there is a real benefit for the mortality rate. In addition, the long-term application of these devices, as for bioreactors, is problematic due to technical limitations and costs.

For this reason, intracorporeal cell-based therapy is one of the most promising technologies under investigation. The article by Navarro-Alvarez and colleagues clearly establishes the clinical advantage of tissue engineering for acute and chronic liver failure, and will be a great help in the designing of routine applications of cell therapies for liver diseases. Naturally, even if this technology offers great promises, the fact remains that there is a need for new active molecules that can be administrated to patients with acute and chronic liver failure in order to promote liver regeneration. The molecular and cellular mechanisms leading to liver regeneration after partial hepatectomy are well characterized, but the mechanisms leading to regeneration in chronic liver diseases remain to be clarified, thus making it difficult to design molecules with therapeutic properties. At present, the only molecule to be recognized and routinely used in clinics against acute fulminant hepatitis is *N*-acetyl-cysteine (NAC). Worldwide, NAC is administrated to patients with acetaminophen-induced fulminant hepatitis and new lines of evidence suggest it has therapeutic properties in non-acetaminophen-induced hepatic failure [21]. There is an urgent need for new therapeutic molecules not only in the treatment of fulminant hepatitis but also in chronic hepatic failure. Until such molecules become available, tissue engineering is a top contender in the list of therapeutic tools for the near future. The rapid progress in our understanding of stem and progenitor cell biology – where important insights have been made into the factors and growth conditions that govern their activation and differentiation into hepatocytes – associated with the recent improvements in tissue engineering technology, may just be the life-saving solution for numerous patients.

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